structural preferences enable promiscuous - yet high affinity - binding to a diverse array of molecular targets.

1627-Pos Board B471
Extreme Mechanical Stability In Polyglutamine Chains Identified Using Single Molecule Force-clamp Spectroscopy
Lorna Dougan, Carmen L. Badilla, Julio M. Fernandez.
Columbia University, New York, NY, USA.
Huntington's disease (HD) is a genetic neurological disorder linked to the insertion of repeats of glutamine (Q) in the protein huntingtin. The increase in the number of Q results in polyglutamine (polyQ) expansions which self-associate to form aggregates. Significantly, there is a strong correlation between the age of onset in HD and the length of polyQ expansions, with postmortem examinations of HD patients identifying large inclusions in the brain. While polyQ aggregation has been the subject of intense studies, very little is known about the structural architecture of individual polyQ chains. An understanding of the molecular properties of polyQ chains is a necessary first step in building a framework to characterize polyQ expansion diseases. Here we demonstrate a single molecule force-clamp technique that directly probes the properties of polyQ. We have constructed polyQ constructs of varying length, namely Q15, Q25, Q50, Q75. Importantly, this length range spans the region where normal polyQ and diseased polyQ expansions have been observed. Each polyQ construct is flanked by the I27 titin module, providing a clear mechanical fingerprint of the molecule being pulled. Remarkably, under the application of force no extension is observed for all lengths of polyQ. We show this is in direct contrast with the random coil protein PEVK of titin which readily extends under force. Our measurements suggest that polyQ form highly stable mechanical structures. We test this hypothesis by disrupting polyQ with insertions of proline residues. Strikingly, upon interruption with prolines the polyQ constructs readily extend under force. These novel experiments provide the first glimpse of the molecular architecture of polyQ expansions, suggesting these structures are mechanically very stable. Such strong structures would be difficult to unravel and degrade in vivo, resulting in polyQ build-up and subsequent aggregation.

1628-Pos Board B472
Small Molecule Binding of Intrinsically Disordered Proteins: Multiple Binders on Multiple Sites
Dalia Hammoudeh[1], Ariele Viachava-Follis[1], Edward V. Prochownik[2], Steven J. Metallo[1].
[1]Georgetown University, Washington, DC, USA, [2]Children's Hospital of Pittsburg, Pittsburg, PA, USA.
We have found that structurally diverse small molecules are capable of specific binding to relatively short segments of intrinsically disordered (ID) proteins. We located such sites on the bHLHZip oncogenic transcription factor c-Myc and on the HLH-only inhibitor of transcription Id2. These proteins are disordered in their monomeric state and only upon dimerization with a partner protein does a stable tertiary structure form. The small molecule inhibitors bind to the ID monomer proteins, affecting their structure at a local level only, preserving the overall disorder and preventing dimerization from taking place.

1629-Pos Board B473
Effect of Vesicle Diameter on α-Synuclein Binding
Elizabeth Middleton, Elizabeth Rhoades.
Yale University, New Haven, CT, USA.
Parkinson's Disease is characterized by the presence of fibrillar deposits of alpha-Synuclein (αS) in the *substantia nigra*. αS is an intrinsically unstructured protein that becomes α-helical upon binding lipid membranes. Many studies indicate that the toxic form of αS may be pre-fibrillar oligomers formed in solution or upon binding to cell membranes or synaptic vesicles. The effect of curvature on αS binding was studied by using Fluorescence Correlation Spectroscopy (FCS) to monitor the binding affinity of αS for synthetic lipid vesicles with different diameters, comparing the wild-type protein with three pathological mutants: A30P, A53T, and E46K. Our findings indicate that bilayer curvature does affect the affinity of αS for net negatively charged vesicles, which may be related to the native function of the protein.

1630-Pos Board B474
Rejuvenation Of CcdB-poisoned Gyrase By An Intrinsically Disordered Protein Domain
Natalie De Jonge, Buts Lieven, Abel Garcia-Pino, Henri De Greve, Lode Wyns, Remy Loris.
VUB, Brussels, Belgium.
Toxin-antitoxin modules are small regulatory circuits that ensure survival of bacterial populations under challenging environmental conditions. The ccd toxin-antitoxin module on the F plasmid codes for the toxin CcdB and its antitoxin CcdA. CcdB poisons gyrase, resulting in inhibition of both replication and transcription. The mechanism by which CcdA actively resolves CcdB:gyrase complexes, a process called rejuvenation, has remained elusive. We have shown that the C-terminal domain of CcdA represents a new class of intrinsically disordered proteins with two distinct but mechanistically intertwined regulatory functions: rejuvenation and transcription regulation. CcdA binds consecutive to two partially overlapping sites on CcdB. This creates two affinity windows that differ by six orders of magnitude and constitutes the key element of a regulatory circuit that links the two functions of CcdA. The first, picomolar affinity interaction triggers a conformational change in CcdB that initiates the dissociation of CcdB:gyrase complexes by an allosteric zipper mechanism. The second, low affinity binding event ensures tightly controlled expression of the ccd operon independent of protection against CcdB activation. The mechanistic complexity of this small network illustrates the potential and versatility of intrinsically disordered proteins for a variety of biological tasks.

1631-Pos Board B475
Fuzzy Complexes: Polymorphism And Structural Disorder In Protein-protein Interactions
Monika Fuxreiter, Peter Tompa.
Institute of Enzymology, BRC, Hungarian Academy of Sciences, Budapest, Hungary.
The notion that all protein functions are determined via macromolecular interactions is the driving force behind current efforts, which aim to solve the structures of all cellular complexes. Recent findings, however, demonstrate a significant amount of structural disorder or polymorphism in protein complexes, a phenomenon that has been largely overlooked thus far. It is our view that such disorder can be classified into four mechanistic categories covering a continous spectrum of structural states from static to dynamic disorder and from segmental to full disorder. To emphasize its generality and importance, we suggest a generic term, 'fuzziness', for this phenomenon. Given the critical role of protein disorder in protein-protein interactions and in regulatory processes, we envision that fuzziness will become integral to understanding the interactome.

1632-Pos Board B476
Collapse Of Rat And Human Amylin From Nanosecond-resolved Intramolecular Contact Formation
Sara M. Vaiana[1], Robert B. Best[2], Wai-Ming Yau[3], William A. Eaton[3], James Hofrichter[3].
[1]Arizona State University, Dept. of Physics, Tempe, AZ, USA, [2]University of Cambridge, Dept of Chemistry, Cambridge, United Kingdom, [3]National Institutes of Health, NIDDK, Laboratory of Chemical Physics, Bethesda, MD, USA.
Amylin is a 37 residue peptide with hormone properties related to nutrient intake regulating glucose levels. It is found in the form of amyloid deposits in the β-cells of type II diabetic patients. Similar to α-syn and Aβ, it is an intrinsically disordered protein. Little is known about amylin's conformational properties in solution and their relation to function and aggregation.
We have used triplet quenching to monitor the dynamics of end-to-end contact formation between the N-terminal disulfide loop of human and rat amylin and a C-terminal tryptophan. The quenching rates for both species increase significantly in aqueous buffer relative to 6M guanidinium chloride (GdmCl), indicating a decrease in the average end-to-end distance. Comparisons with control peptides suggest that backbone-backbone interactions, involving the N-terminal disulfide loop are the principal driving force for collapse in these peptides, rather than sidechain-sidechain hydrophobic interactions. Molecular dynamics simulations on the control sequences indicate that the collapse results from hydrogen-bonding interactions between the central residues of the chain and the disulfide loop, reducing the length of the free chain by ~ 2-fold. This structural feature may contribute to the functional role of the disulfide loop in amylin and in the larger family of calcitonin gene-related peptides. We discuss the newly observed differences between monomeric human and rat IAPP in solution and their possible relation to aggregation.

1633-Pos Board B477
Conformational dynamics of titin PEVK explored with FRET spectroscopy
Tamas Huber[1].
University of Pecs, Pecs, Hungary.
Titin's PEVK domain, which is responsible for the molecule's physiological extensibility, is thought to be an instrinsically unstructured protein region. The structural dynamics, induced conformations, and interactions of the PEVK domain are far from being fully understood.